Local-scale diversity and adaptation along elevational gradients assessed by reciprocal transplant experiments: lack of local adaptation in silver fir populations

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Local-scale diversity and adaptation along elevational gradients assessed by reciprocal transplant experiments: lack of local adaptation in silver fir populations

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Abstract

• *Key message* Silver fir transplantations along elevational gradients revealed a high diversity but no local adaptation. Populations displayed similar abilities to adapt to new environments including those due to climate change.
• *Context* The sustainability of forest stands depends on the ability of species and communities to adapt by combining plasticity and genetic evolution. Although well-documented at the scale of species distributions, the variability and adaptation of forest tree genetic resources are less understood at the short-distance scale.
• *Aims* We analysed the effects of genetic and environmental factors on the local-scale phenotypic diversity of traits related to adaptation in *Abies alba*. We also sought to highlight local adaptation, revealing past selection.
• *Methods* Six adaptive traits related to growth, phenology and survival were measured on seedlings from 57 half-sib families collected from 15 provenances and planted in a nine-site reciprocal transplant experiment distributed along three elevational gradients.
• *Results* Most part of the phenotypic variability was attributed to the environmental factors. Provenances and families had also significant effects on seedling performances, but the genetic variability was mostly attributed to the families. No pattern of local adaptation was observed, except in the presence of lateral branches in the driest garden.
• *Conclusion* The absence of local adaptation suggests a similar ability of all silver fir populations to develop in the various environments. This result provides favourable conditions for coping with the ongoing climate change without exotic resources enrichment.

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1 Introduction

To cope with the variations in their environments, populations of living organisms engage (i) plastic response (i.e. adjusting their phenotypes), (ii) migration and (iii) genetic adaptation through selection (Jump and Peñuelas 2005; Aitken et al. 2008). The ongoing and rapid climate change will undoubtedly be a major driver modifying habitat features. Long-lived species, such as forest trees, will be more severely affected. It is now broadly recognised that the velocity of climate change will surpass most tree migration rates (Davis and Shaw 2001). Thus, phenotypic plasticity and genetic variability will be of major importance in determining species potential for adaptation. Tree distribution areas are often wide, and most natural selection pressures, therefore, vary across the species range. The scales at which gene

flows and natural selection act (Boshier et al. 2015) shape population diversity and differentiation.

Common garden studies conducted on forest tree provenances originating from long-distance elevational or latitudinal gradients revealed that traits related to growth, phenology and cold hardiness present significant differences between populations and are correlated with climatic characteristics (mostly temperature) of the site of origin (reviewed in Aitken et al. 2008, Alberto et al. 2013, Savolainen et al. 2007). In some cases, this results in local adaptation, i.e. better performance of the local population (Kawecki and Ebert 2004). Some studies conducted at the local scale have shown that plants are phenotypically and genetically differentiated over short-distance gradients (Isik and Kara 1997; Sáenz-Romero et al. 2006; Byars et al. 2007; Gonzalo-Turpin and Hazard 2009). However, the relationships between the performance of the populations (assessed on survival and on functional, reproductive or phenology-related traits) and the environmental conditions of their location of origin are not always consistent (Isik and Kara 1997; Lara-Romero et al. 2014; Frei et al. 2014).

The present study aims to evaluate the genetic diversity and adaptation of silver fir (*Abies alba* Mill.) at the scale of three elevation transects using a multi-site reciprocal transplant experiment of seedlings. The gradients are located in the French Mediterranean region, at the southern limit of the natural range of *A. alba*, on Mont Ventoux, Issole and Vésubie mountains. These mountains are representative of the inland region of southeastern France. During the nineteenth century, overexploitation of forests and the pressures of pastoralism led to deforestation of the majority of these areas (Cailleret and Davi 2011). In the late nineteenth and early twentieth centuries, the decrease in grazing associated with an active afforestation policy allowed large-scale restoration of forest cover. The afforested stands were then naturally recolonised by postpioneers (*Sorbus aria* and *Acer opalus*) and shade-tolerant species (*Abies alba* and *Fagus sylvatica*). Our experiment focused on silver fir, *A. alba*, a keystone species (Saccone et al. 2009) found at the end of ecological successions. On most European mountains, *A. alba* can be found from 400 to 1800 m above sea level (Aussenac 2002).

Silver fir is a shade-tolerant (Saccone et al. 2009), drought sensitive (Aussenac 2002; Lebourgeois et al. 2010) and requires high atmospheric humidity due to its high sensitivity to summer drought (Lebourgeois 2007). The expected increase in the frequency of drought events (IPCC 2013) makes the silver fir a model species for climate susceptibility for the purpose of assessing the response of long-lived organisms to climate change, especially at the xeric edge of the species range.

Based on a reciprocal transplant experiment conducted on silver fir seedlings, the study addresses the two following questions: (i) what are the contributions of the genetic and environmental factors (and their interaction) to the phenotypic variability of traits related to adaptation and (ii) does local adaptation occur along elevational gradients? Results will provide information on natural silver fir population dynamics and basis for the forest management recommendations.

2 Methods

2.1 Study sites

A multi-site reciprocal transplant experiment was conducted over three mountains located along a 200-km west-to-east longitudinal gradient from the Rhône Valley to the Alps (Fig. 1): Mont Ventoux, Issole and Vésubie. These mountains belong to the three ecological groups (intermediate Alps, sub-Mediterranean Alps and Ligurian Alps; Sagnard et al. 2002) and present slightly contrasting climatic conditions: higher temperature on Vésubie (+ 1.3 °C) and higher precipitation on Mont Ventoux (1126 mm per year) than on Vésubie (954 mm) or Issole (856 mm; Cailleret et al. 2014). The soils are shallow (30 to 70 cm) but exhibit rather high variability in depth and texture between and within sites. The bedrock is a sandstone schist on Vésubie, a calcareous clay on Issole and a calcareous karst on Mont Ventoux. The soil water content is low and varies according to the proportion of coarse fragment content (30 to 90%) and the depth of the bedrock.

2.2 Seed-tree sampling and seed collection

Populations of firs were sampled from throughout the elevation range of the species on the northern slopes of the three gradients. Open-pollinated seeds were collected in 2008 from 84 seed trees selected at five elevations (referred to as "provenances"; Fig. 1) of the three mountains hereafter referred to as the "mountains of origin". The geographic distances between the lowest and the highest provenances were 1.5 km for Mont Ventoux and Issole and 2.5 km for Vésubie. Hereafter, the provenances will be referred to using a combination of the first three letters of the mountain name and the provenance elevation rank (e.g. "Iss1" for the lowest elevation provenance at Issole). Based on the availability of viable seeds, four seed lots were selected per provenance, except for Ves1 (two) and Ves3 (three), leading to a total of 57 seed lots.

2.3 Reciprocal transplant design

Before being transferred to the forest sites, the seedlings were raised at the state forest nursery of Aix-Les-Milles in the spring of 2009. To limit transplantation stress and reduce the effect of soil variability between sites, the seedlings were raised in wood vegetable crates (30 × 50 cm), homogeneously
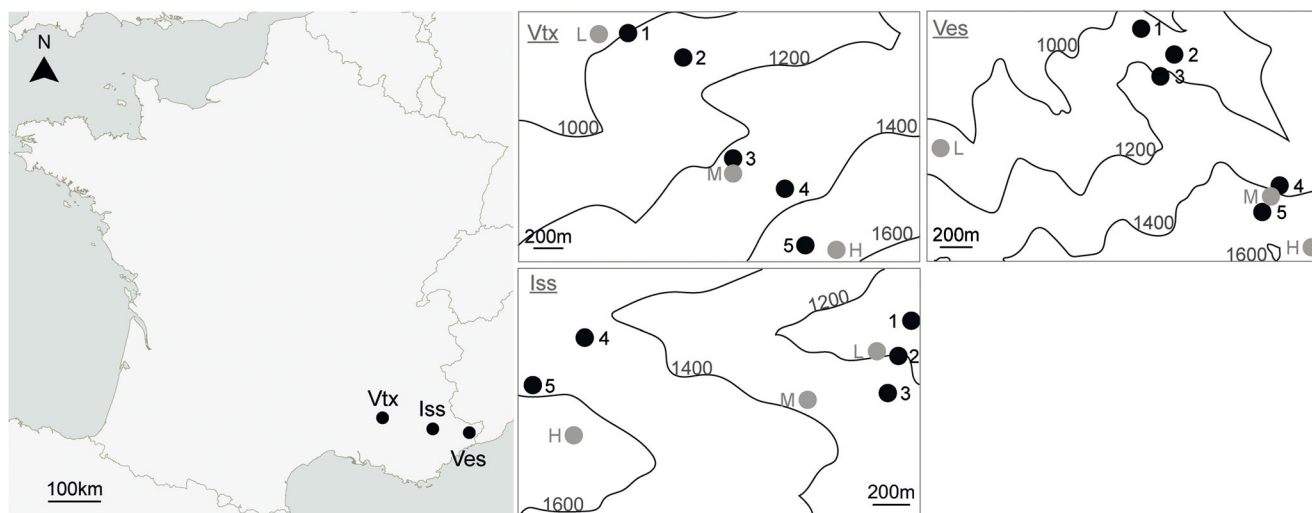
Fig. 1 Mountain locations and schematic representation of the location of each provenance (black circles) and common garden (grey circles). 1 to 5: low to high provenances. L = low, M = mid, H = high. The grey numbers correspond to the elevation (in meters asl)

filled with a mixture of peat and bark. The bottoms of the crates were replaced with wire mesh to favour full soil contact and allow free root growth in the soil.

The design of the reciprocal transplant experiment was already set up at the nursery stage. It consisted of nine replications (one per transplantation site) of a complete block experiment. Each replication included five blocks of 12 crates containing five rows of seven seedlings from the same half-sib family. Each row will be referred to as a "unit plot" (UP). Families were randomly distributed within blocks.

Due to the low germination rates of some seed lots, the overall experiment included 14,383 seedlings instead of 17,955 (57 seed lots × 7 seedlings × 5 blocks × 9 replications).

In the early fall of 2009, the common gardens, one per replication, were installed at three elevations (low, middle and high) on each mountain (Fig. 1). The locations of the common gardens were representative of the environmental conditions of the adult tree provenances and exhibited mid-level canopy openings. Hereafter, each common garden will be referred to using a combination of the first three letters of the mountain name and the initial letter of the elevation (e.g. "Iss_L" for Issole, low-elevation).

The crates were buried in the forest soil, and the seedlings were protected from grazing by a fence.

2.4 Environmental data

Daily air temperatures and the relative air humidity of the forest provenances and common gardens were collected using HOBO Pro V2 micro-loggers (Onset Computer Corporation, Cape Cod, Massachusetts, United States). The climates of the gardens and of the sites of origin of the provenances were

characterised by the annual mean values over the 2009 to 2015 period.

2.5 Seedling phenotype measurements

We measured fitness-related traits related to survival, growth and phenology. Bud burst was chosen as the phenological variable. It determines the length of the growing season and is related to the risk of injury from spring frost. The bud burst score, derived from the BBCH-scale (Finn et al. 2007), ranged from 0 for dormant buds to 10 for mature needles. Seedling growth was assessed based on four traits: (i) the height increment, (ii) the presence of lateral branches, (iii) the length of the longest lateral branch and (iv) total dry biomass. Lateral branches are involved in the acclimatisation to shade (Metslaid et al. 2007), highly variable and under genetic control (Wu and Hinckley 2001).

The seedling survival rate, as well as the presence and length of lateral branches, were recorded in 2013. The height increment was computed as the difference between the 2013 and 2010 heights, removing growth in the nursery. Bud burst was scored over 3 years (2011 to 2013) through two observations conducted 2 weeks apart in April or May. The analyses were carried out using either single bud burst observations or summed bud burst across observations (2) and years (3). All analyses led to very similar results, and only the results from the summed data are presented. In addition to the direct measurement of seedling growth (height and branch), we estimated the total biomass as a synthetic trait integrating seedling height and architecture. As biomass can only be measured from destructive sampling, a model was calibrated using a subset of seedings

harvested in 2014 from four gardens (Iss_high, Iss_low, Vtx_high and Vtx_low; two blocks per garden).

2.6 Statistical analyses

2.6.1 Estimation of seedling total biomass

To estimate seedling biomass, we first fitted two regression models (*lm* function, R Core Team 2016) onto the harvested seedlings—one for seedlings without lateral branches (Eq. 1) and one for seedlings with lateral branches (Eq. 2).

$$TW_i = \beta \times Height_i + \alpha \qquad (1)$$

or

$$TW^{1/2}{}_i = \beta \times Height_i + \gamma \times LB_i + \alpha \qquad (2)$$

where TW = total weight and LB = length of the longest branch.

2.6.2 Phenotypic variability of seedlings

A nested ANOVA (McDonald 2009) model was used to analyse the variability of the traits, according to genetic and environmental factors (Eq. 3).

$$Y = Or + Pr/Or + F/Pr/Or + Pl + CG/Pl + B/CG/Pl$$
$$+ UP + \Sigma\ interactions + \varepsilon \qquad (3)$$

where Or = mountain of Origin, Pr = provenance, F = half-sib family, Pl = mountain of plantation, CG = common garden, B = block, UP = unit plot and interactions = all the environment × genotype interactions (Online Resource 1), ε = residual.

Environmental factors reflected seedling phenotypic plasticity, while genetic factors reflected variability due to their origin. The interactions between environmental and genetic factors reflected the variation of phenotypic plasticity due to the seedling origin. Both types of factors corresponded to three-level nested designs: half-sib families within provenances within the mountains of origin and blocks within common gardens within the mountains of plantation. Provenances and gardens were analysed as fixed effects because they were selected for the purpose of the experiment and covered the entire elevational range of the species. Family and all family-by-environment interactions were random effects (Online Resource 1).

Height increment, total weight, length of the longest lateral branch and bud burst were analysed using a linear model (*lm* function from *R*). Height increment and length of the longest branch were root-square transformed, and biomass was log transformed. The presence of lateral branches and the survival rate were analysed using a generalised linear model with the logit link function for binomial variables. We used the

Bayesian method (*bayesglm*) available within the arm package (Gelman et al. 2009) to overcome the complete separation problems that can occur for the most nested factors (i.e. family and block), and the absence of overdispersion was checked. Analyses were run with the following constraints: (i) $\sum F_i = 0$ for fixed factors and (ii) $F_i \sim N(0, \sigma^2)$ for random factors, where F_i is the ith level of factor F.

In addition to the ANOVA results, the contribution of each factor to the overall variability was computed from the expected mean square (Online Resource 1). The variability due to each random factor was its variance (σ^2). The variability due to each fixed factor was estimated as the variance of the random variable exhibiting a discrete and equiprobable distribution with a number of categories equal to the number of levels of this factor. In this context, the variance of the discrete variable is computed as $\sum(X_i - X_{mean})^2/N$.

2.6.3 Local adaptation

We assessed the local adaptation by analysing the relation between family performances and environmental distances. When locally adapted, a family performs better when planted in environmental conditions close to the environmental conditions of its seed-tree location. The performance of each family was thus analysed according to the climatic distance, computed as the difference in temperature (ΔT) or in vapour pressure deficit (ΔVPD) between the seed tree environment and the common garden environments. The adaptation of a tree to its local environment is likely to occur over a longer climatic period than the one studied here. However, if the climate has evolved during the seed-tree life, we assume that the climatic distances between the two locations (i.e. seed-tree and common garden) have not changed.

For clarity, ΔT and ΔVPD will subsequently be referred to as "climatic distance" and temperature and vapour pressure deficit to as "climatic conditions". The symmetry of the family performances relative to the zero climatic distance was checked. When symmetric (i.e. warmer and colder conditions have similar effects), the link between the climatic distance and the family performance was estimated using the absolute value of the distance. This link was assessed using linear regression models. Non-linear models were also tested but did not lead to better fits. In a first step, we tested the "home vs. away" criterion of local adaptation. The relationship between family performance and climatic distance was estimated by provenance. Thus, the performance of families planted under climatic conditions close to those of their provenance was compared to the performance of the same families planted under distant climatic conditions. In a second step, we tested the "local vs. foreign" criterion of local adaptation. The relationship between family performance and climatic distance was estimated by garden. Here, the performance of families planted under climatic conditions close to those of their

original provenance was compared only with the performance of families originating from distant climatic conditions. To conclude that families or gene pools associated with each provenance are locally adapted, both criteria ("home vs. away" and "foreign vs. local") must be met.
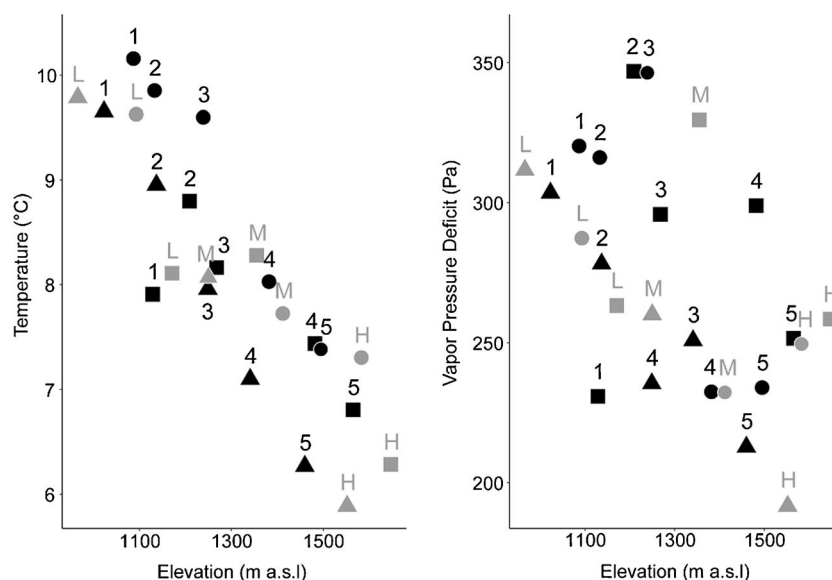
3 Results

3.1 Environmental characteristics

As expected, thermal gradients were observed along the elevational gradients. The temperature decreased regularly with elevation by 0.73, 0.50 and 0.69 °C/ 100 m for Mont Ventoux, Issole and Vésubie, respectively, (Fig. 2). The vapour pressure deficit also tended to decrease with elevation, but the relationship is less strong than for temperature. Over the 2009 to 2015 period, Vésubie was 1 °C warmer than both other sites (Fig. 2). Due to their location at the bottom of the valley, Iss1 and Iss_L exhibited a lower vapour pressure deficit and lower temperatures than expected. Moreover, Ves_H was 1.2 °C warmer than Iss_H and Vtx_H. Ves_H is located close to the crest and could be influenced by warm air coming from the southern exposure.

3.2 Seedling biomass

The combinations of height increment and the length of the longest branch provided good estimators of the total biomass ($R^2 = 0.8$, Online Resource 2). These models were used to estimate the biomass of the other seedlings.

3.3 Average trait performance and variability

The overall survival rate decreased gradually from 98 to 78% between 2010 and 2013 (Online Resource 3). The seedling total height, presence of lateral branches and length of the longest branch increased almost linearly with years. In 2013, the mean seedling height was 6.9 cm, and 70% of the seedlings exhibited branches, with a length of the longest branch that averaged 2.9 cm. For 24% of the seedlings, the branch length surpassed the total height—for 22.5%, it was twice the total height.

3.4 Structure of phenotypic variability

The ANOVA model (Eq. 3) explained 37 to 90% of the variability of the analysed traits (Fig. 3).

3.4.1 Phenotypic plasticity

For all traits, the variability explained was mostly attributed to environmental factors (65% for the presence of lateral branches to 98% for bud burst). For growth (height increment, presence of lateral branches, branch length and biomass) and survival, 61% of the environmental variability was due to the micro-environment (blocks and unit plots). The remaining environmental variability was primarily due to the mountain of plantation for total weight, branch length and survival (on average, 35%). Indeed, seedling biomass was globally lower (− 0.18 g), and lateral branches were shorter (− 1.41 cm) on Vésubie (Online Resource 4). Moreover, survival was 28% higher on Mont Ventoux than on Issole and Vésubie. On the other hand, for height increment and the presence of branch, the remaining environmental variability was primarily due to the common garden (on average, 26%; Fig. 3). Indeed, the

Fig. 2 Elevational and climatic characterisation of the provenances and common gardens involved in the *A. alba* nine-site reciprocal transplant experiment: plot of mean annual temperature (left side) and vapour pressure deficit (right side) versus elevation. Provenances are represented by black symbols (1 to 5 for low to high elevations) and common gardens by grey symbols (L = low, M = mid, H = high) and triangles: Mont Ventoux, squares: Issole and circles: Vésubie
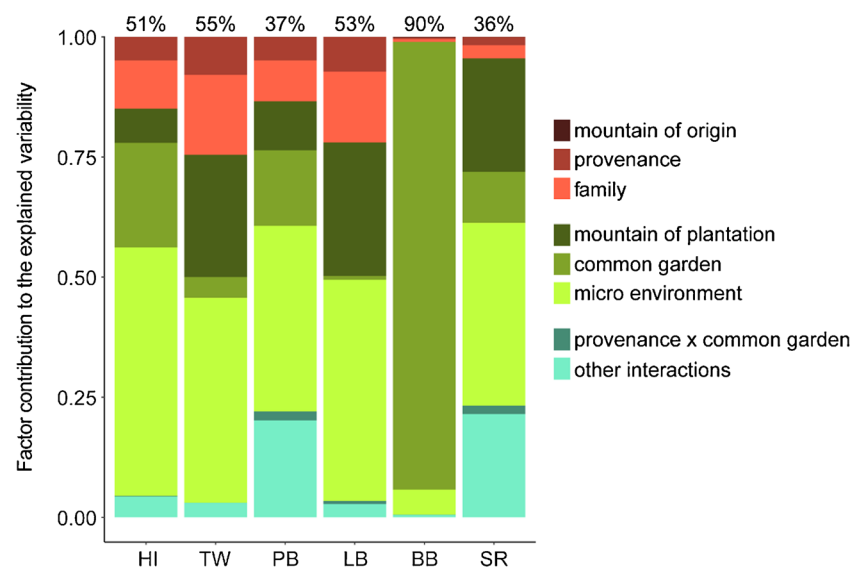
Fig. 3 Contribution of the experimental factors to the variability explained by the model for growth, phenology and survival measured in 2013 in the *A. alba* nine-site reciprocal transplant experiment. The above bar plot values represent the proportion of total variance explained by the model (Eq. 3). Factor and interaction ordering is the same for every bar plot, but some values are too low to be visible (e.g. mountain of origin). HI = height increment; TW = estimated total dry weight; LB = length of the longest branch; BB = bud burst phenology; PB = rate of presence of lateral branches; SR = survival rate

presence of branch was lower by 15, 54 and 17% in Iss_H, Vtx_H and Ves_M, respectively, than in the other gardens. Height increment was also lower in Ves_M than in Ves_H and Ves_L by 0.33 cm, greater in Iss_H than in Iss_L and Iss_M by an average of 1.4 cm and equivalent for the three gardens of Mont Ventoux (approximately 2.8 cm; Online Resource 4).

The environmental variance of bud burst was mostly explained by the garden (95%), whereas very little variance was explained by micro-environmental factors (5%, Fig. 3). Although the between mountain variability of bud burst was not significant as compared to the variability between gardens, seedlings burst 13 days later on average on Issole than on both other mountains, due to the temperature inversion on Issole (Iss_L burst later than expected). Regardless of the plantation mountain, bud burst was always significantly earlier in gardens with higher temperatures (Vtx_L, Ves_L and Iss_M). The first needles reached their adult stage in Vtx_L on the same date when buds began to inflate in Vtx_H which corresponds to a delay of 3 weeks.

3.4.2 Variability due to the origin of the seedlings

Genetic factors explained 19% of the explained growth variability but only 4% of the survival variability on average (Fig. 3). Despite this difference, the relative proportion of variance explained by each of the genetic factors was similar for all of the growth, phenological and survival-related traits: close to zero (2% on average) and not significant for the mountain of origin factor, 34% on average for the provenance factor and 64% on average for the family factor. Seedlings originating from the

warmest provenances of the three mountains (Iss2, Ves1 and Ves2, and Vtx1) survived better (+ 8.2%), produced branches more frequently (+ 25%) and exhibited longer branches (+ 0.9 cm), greater growth (+ 0.5 cm) and greater biomass (+ 0.14 g) than seedlings originating from the other provenances (Fig. 4). Seedlings originating from the warmest provenances burst 2 to 4 days before the other origins.

There was no significant genotype-by-environment interaction (G×E) for height or presence of lateral branch (Table 1). The interaction between families and common gardens explained three and 0.6% of the variability of biomass and bud burst, respectively, (*p* values < 0.025; Table 1). Various G×E interactions, including provenances by common gardens, explained 23% of the variability of the presence of branch and survival on average.

3.5 Evaluation of local adaptation

According to the "local vs. foreign" criterion, seedlings originating from provenances where the temperature was close to those of the Ves_L and Vtx_L common gardens (i.e. the warmest common gardens) tended to burst earlier and to exhibit higher values for biomass and lateral branch length when growing in those common gardens (Table 2). Similarly, seedlings originating from provenances where vapour pressure deficit was close to that of the Vtx_L common garden (high VPD) exhibit higher values for these traits and produce more branches. This higher rate of lateral branch production in dry air conditions is also observed for seedlings evaluated in Iss_M which is the common garden with the highest vapour pressure deficit (Table 2, Online Resource 6). The "home vs. away" criterion is only met for the production of lateral

Fig. 4 Average predicted performances of the families originating from the different provenances (1 to 5) of each of the three mountains (orange = Mont Ventoux; red = Issole; blue = Vésubie). The performances were computed over all the nine common gardens. HI = height increment; TW = estimated total dry weight; LB = length of the longest branch; BB = bud burst phenology; PB = rate of presence of lateral branches; SR = survival rate
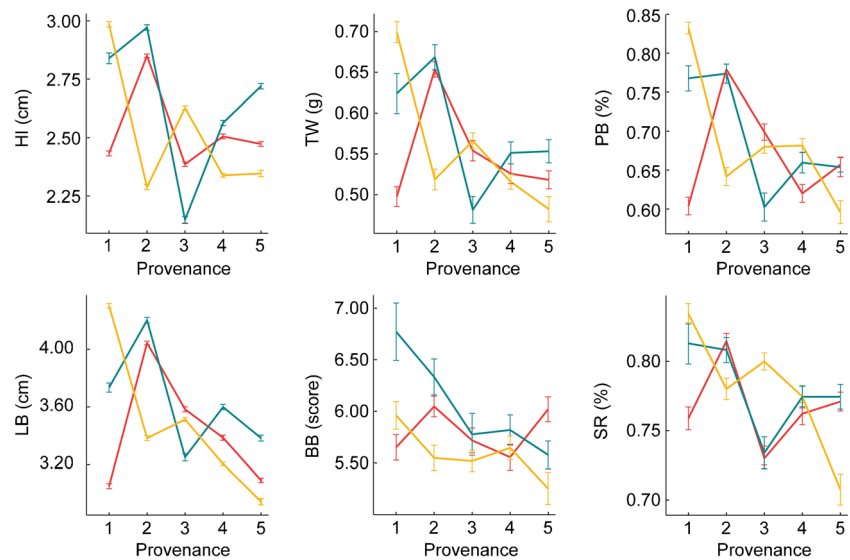
branches in seedlings from Iss2, the provenance originating from the highest vapour pressure deficit conditions (Table 2). Thus, we can conclude that strict local adaptation (i.e. where both criteria were met) would only occur for lateral branch production and for seedlings from the driest site (see Online Resource 6 for regression plots for the Iss2 provenance and the Iss_M common garden). There was no evidence of local adaptation for temperature, and the highest survival rates of the seedlings originating from the lowest elevated provenances suggest the absence of a specific adaptation to frost.

Table 1 Variance (deviance for binary traits) analysis of the growth, survival and bud burst phenology of silver fir seedlings in the *A. alba* reciprocal transplant experiment. *p* values were computed from F-tests of mean squares ratios for linear models and from Chi-square deviance tests for GLM (Online Resources 1 and 5)

Factors[a]	Type[b]	*p* values[c]					
		HI	TW	LB	BB	PB	SR
Or	G	0.69	0.92	0.90	0.22	0.23	0.06
Pl	E	0.24	**0.01**	**0.002**	0.46	**$<10^{-16}$**	**$<10^{-16}$**
Provenance	G	**0.004**	**0.004**	**0.001**	**0.006**	**$<10^{-16}$**	**$<10^{-13}$**
C.garden	E	**$1.72\ 10^{-5}$**	**0.04**	0.30	**$<10^{-16}$**	**$<10^{-16}$**	**$<10^{-16}$**
Family	G	**$<10^{-16}$**	**$<10^{-16}$**	**$<10^{-16}$**	**$<10^{-16}$**	**$<10^{-16}$**	**$<10^{-14}$**
Block	E	**$<10^{-16}$**	**$<10^{-16}$**	**$<10^{-16}$**	**$<10^{-16}$**	**$<10^{-16}$**	**$<10^{-16}$**
Unit Plot	E	**$<10^{-16}$**	**$<10^{-16}$**	**$<10^{-16}$**	**$<10^{-16}$**	**$<10^{-16}$**	**$<10^{-16}$**
Or × Pl	G×E	0.26	0.59	0.99	0.42	0.63	0.68
Or × C.garden	G×E	0.42	0.18	0.12	0.11	0.08	0.05
Or × Block	G×E	0.20	0.16	0.11	0.79	**0.03**	**0.004**
Provenance × Pl	G×E	0.31	0.32	0.64	0.22	**0.002**	0.45
Provenance × C.garden	G×E	0.37	0.47	0.12	0.23	**0.01**	**0.01**
Provenance × block	G×E	0.91	0.99	0.97	0.29	0.94	0.05
Family × Pl	G×E	0.34	0.69	0.15	0.83	0.18	**$<10^{-04}$**
Family × garden	G×E	0.05	**0.02**	0.35	**$4.8\ 10^{-04}$**	**$<10^{-04}$**	**$<10^{-07}$**

Significant *p* values (significance threshold of 5%) are shown in bold

[a] *Or* mountain of origin, *Pl* mountain of plantation, *C.garden* common garden

[b] *G* genetic factor, *E* environmental factor, *G×E* genotype-by-environment interaction, *HI* height increment, *TW* estimated total dry weight, *LB* length of the longest branch, *BB* bud burst phenology, *PB* rate of presence of lateral branches, binary trait, *SR* survival rate, binary trait

Table 2 Slopes of the regressions between the performance of the seedlings and the climatic distance. The climatic distance is the absolute value of the difference of the temperatures (°C) or vapour pressure deficit (kPa) between the site of origin (provenance) and the site of the test (common garden). Negative coefficients correspond to greater performance at home than elsewhere ("home vs. away" criterion) or greater performance of the local seedlings than the foreign ones ("local vs. foreign" criterion)

		HI		TW		LB		PB		BB		SR	
Criterion	Provenance	ΔT	ΔVPD	ΔT	ΔVPD	ΔT	ΔVPD	ΔT	ΔVPD	ΔT	ΔVPD	ΔT	ΔVPD
Home vs. Away	Vtx1	− 0.03	− 0.04	− 0.02	0.01	− 0.02	0.06	− 0.15	− 0.27	− 0.14	− 0.45	0.19	− 0.07
	Vtx2	0.09	0.08	0.10	0.14	0.09	0.10	− 0.02	0.89	0.37	0.62	− 0.42	1.07
	Vtx3	0.01	0.05	0.01	− 0.01	− 0.05	− 0.26	0.37	1.71	− 0.05	− 0.16	0.39	− 0.77
	Vtx4	0.03	− 0.01	> − 0.01	− 0.12	− 0.04	− 0.11	− 0.08	− 1.05	− 0.03	− 0.27	0.25	− 0.35
	Vtx5	− 0.04	− 0.10	− 0.07	− 0.21	0.15	0.33	− 0.05	− 1.85	− 0.14	0.40	− 0.24	− 0.39
	Iss1	− 0.04	− 0.04	− 0.04	− 0.11	− 0.03	− 0.31	0.11	0.20	> − 0.01	− 0.15	− 0.52	− 0.21
	Iss2	− 0.02	− 0.02	− 0.05	− 0.08	> − 0.01	− 0.01	− 0.54	**− 1.68**	− 0.17	− 0.22	0.11	0.20
	Iss3	− 0.12	− 0.06	− 0.16	− 0.07	− 0.15	0.16	− 0.43	0.30	− 0.46	− 0.16	0.13	− 0.41
	Iss4	0.01	− 0.11	0.03	− 0.17	0.12	− 0.08	0.50	− 1.14	< 0.01	− 0.08	− 0.06	0.06
	Iss5	− 0.01	0.11	> − 0.01	0.09	− 0.09	0.05	0.55	1.36	− 0.06	− 0.44	0.06	− 1.05
	Ves1	0.02	0.03	0.01	0.02	> − 0.01	− 0.02	0.22	0.89	0.02	0.04	0.42	1.52
	Ves2	0.02	0.06	0.03	0.12	0.03	0.13	− 0.13	0.10	− 0.05	− 0.16	− 0.12	− 0.54
	Ves3	− 0.04	− 0.01	− 0.02	0.03	− 0.24	− 0.29	− 0.20	− 0.98	− 0.06	0.03	− 0.22	0.32
	Ves4	− 0.04	− 0.27	− 0.04	− 0.26	0.10	− 0.21	− 0.45	− 0.64	0.17	− 0.08	− 0.17	1.06
	Ves5	− 0.03	− 0.04	− 0.02	− 0.05	0.02	− 0.01	0.32	0.81	0.01	0.10	0.33	1.68
	Common garden												
Local vs. Foreign	Vtx_L	− 0.02	− 0.14	**− 0.07**	**− 0.31**[a]	**− 0.09**	**− 0.34**[a]	− 0.33	**− 1.76**	**− 0.20**	**− 0.63**	− 0.15	− 0.75
	Vtx_M	− 0.06	0.08	− 0.07	0.17	0.23	0.26	− 0.35	2.18	0.05	1.11	− 0.24	2.56
	Vtx_H	0.01	− 0.02	0.08	0.12	0.14	0.18	0.62	0.80	0.04	0.02	0.08	− 0.04
	Iss_L	− 0.10	0.12	− 0.05	0.38	0.17	0.41	0.62	1.03	− 0.01	0.54	0.07	− 0.46
	Iss_M	− 0.12	− 0.27	− 0.25	− 0.56	− 0.21	− 0.66	− 0.98	**− 3.79**[a]	− 0.92	− 1.30	− 1.10	0.22
	Iss_H	0.02	0.08	0.05	0.15	0.05	0.16	0.27	0.72	0.03	0.19	− 0.13	− 0.84
	Ves_L	− 0.05	− 0.09	**− 0.08**[a]	− 0.20	**− 0.18**	0.62	− 0.54	− 1.89	**− 0.18**	0.08	− 0.31	− 0.88
	Ves_M	0.10	0.10	0.15	0.15	0.03	0.06	1.54	1.70	0.45	0.52	0.19	− 0.08
	Ves_H	0.07	0.14	0.09	0.20	0.09	0.23	0.53	1.07	0.19	0.56	0.46	1.14

Bold font for significant p values (i.e < 5%)

HI height increment, *TW* estimated total dry weight, *LB* length of the longest branch, *BB* bud burst phenology, *PB* rate of presence of lateral branches, *SR* survival rate

[a] for coefficient remaining significant after Bonferroni correction per variable. Due to the limited number of points contributing to each regression, none of the values remained significant when adjusted for all the regressions

4 Discussion

4.1 Environmental and genetic differentiation between populations

This multi-site reciprocal transplant experiment allowed us to highlight a significant structure of the phenotypic variability attributed to both the environmental and genetic factors. Similar results are commonly obtained for short-distance elevation transects in tree species (see 'Introduction' section) including silver fir (Sagnard et al. 2002; Robakowski et al. 2005) for many morphological, phenological and life-history traits. According to Bischoff et al. (2006), inter-population

variability can be higher between geographically close populations experiencing contrasting conditions than between distant populations. At a short-distance scale within our gradients, both phenotypic plasticity and genetic variability contributed to the adaptive potential of silver fir, except with regard to the bud burst phenology in which variability was related mainly to the common garden temperature. As previously observed in most tree species examined to date (e.g. Mimura and Aitken 2010; Vitasse et al. 2013), bud burst occurs earlier in warmer common gardens. Contrary to our results, bud burst is also expected to be subject to greater genetic control than growth-related traits (Aitken et al. 2008). Vitasse et al. (2009) already reported a low level of variability of bud

burst between *Abies alba* populations but did not provide any information about within-population variability and genetic control of the trait. Our results suggest that as an evergreen tree, silver fir is less susceptible to early unfolding than deciduous trees. Indeed, its early photosynthetic activity not only depends on the development of new leaves but largely relies on the pre-existing foliage. Studying the relationships between the timing of budburst and various plant traits in 24 coexisting deciduous or evergreen broadleaf species, Osada (2017) observed that, in evergreen species, budburst was not correlated with any of the plant traits analysed. Moreover, budburst timing was significantly earlier for the deciduous. Thus, selection pressure on budburst might be more severe in deciduous than in evergreen species.

In a previous single-site common garden experiment comparing half-sib families from different fir populations (i.e. provenances) from the southwestern Alps, Sagnard et al. (2002) also highlighted high intra-population variances for a large set of traits (e.g. growth, bud phenology and drought resistance). Within each of our nine common gardens, the intra-population (i.e. inter-family) variance was 1.5 to 2 times higher than the inter-population variance. We can presume that the high within-population genetic variability mainly results from the high heterogeneity of local environments.

The significant differentiation of silver fir populations observed along short-distance gradients supports the hypothesis that gene flows are not sufficient to fully homogenise genetic pools. More than the distance between trees, the large shift in phenological stages due to the climate differences between elevations ($\Delta T \sim 4\ °C$), does not allow for cross-fertilisation among all the trees distributed along the elevation gradients. On the other hand, the lack of differentiation among silver fir seedlings originating from different mountains suggests the occurrence of long-distance pollen flows and cross-mountain fertilisation of trees exhibiting synchronised flowering periods. This last hypothesis is strengthened by the low genetic differentiation among the three mountains, as observed using SNP markers in adult trees from the same mountains (Roschanski et al. 2016).

4.2 Phenotypic variation between populations is independent from the climate at origin

Although significant differences were observed between seedlings of origins located at different elevations, there were no consistent trends related to temperature variations or vapour pressure deficit among the elevational origins. This result is consistent with previous observations made in silver fir at a long-distance scale (Robakowski et al. 2005). Clines of genetic variability are frequently observed along large geographic distance but far less often along short-distance gradients. In the present study, the absence of correlations between seedling performance and the climatic conditions of the seed tree

provenances suggests that other climatic or micro-environmental factors exhibiting variations unrelated to elevation may contribute to the population genetic differentiation. Specifically, environmental conditions (e.g. soil depth, slope, water reserves), which are highly variable at the micro-local scale on these Mediterranean mountains, could explain the absence of any coherent differentiation of populations along the elevational gradients. A low climate-related differentiation among fir populations was also observed by Frank et al. (2017) in a one site common garden where populations of silver fir and Norway spruce collected across most of Switzerland were evaluated. Thus, fir acts as an adaptive generalist and compared to spruce that is an adaptive specialist.

Although no general trends were observed, the fir seedlings originating from the warmer provenances exhibited better global performance, both "at home" and "away". This finding has been reported previously in other species, at both a short-distance scale along an elevation transect (e.g. for phenology traits in *Quercus oleoides*; Deacon and Cavender-Bares 2015) and a long-distance scale (e.g. for various forest trees and for growth and adaptation to frost-related traits; Kreyling et al. 2014, Thiel et al. 2014).

4.3 Local adaptation is only detected in populations from dry origin

Our "local vs. foreign" evaluation of adaptation indicates that growth-related traits and bud burst would be positively selected in populations from the warmer origins, but the "home vs. away" criterion does not indicate a pattern of local adaptation. The provenance × elevation interactions resulted in a significant pattern of local adaptation for only one low-elevation population and one trait—seedlings originating from locations with a high vapour pressure deficit produced more lateral branches. The relationship between vapour pressure deficit and the presence of branches is unclear and, to our knowledge, is not discussed in the literature. We suggest that the presence of lateral branches could create a plant micro-climatic environment that would be favourable under dry conditions. This hypothesis has to be tested in other experiments preferably in controlled conditions where seedlings would be raised in an environment fully qualified.

In all other cases, the absence of local adaptation is coherent with previous results reviewed by Leimu and Fischer (2008) showing for plants species that the criteria for local adaptation are met more rarely than expected. Over both short- and long-distance gradients, evidence of local adaptation depends on the criterion selected to define it. Indeed, the strict definition of local adaptation, taking into account both the "home vs. away" and "local vs. foreign" criteria, is rarely met. Kawecki and Ebert (2004) suggest that if only one of the two criteria must be taken into account, it is better to consider the "local vs. foreign" criterion. Indeed, this criterion directly

reflects the main force leading to local adaptation—divergent natural selection. In contrast, the "home vs. away" criterion confounds the effects of divergent selection and the intrinsic differences in environmental quality. In the present study, the populations that met the "local vs. foreign" criterion not only performed better at home than the other populations, they also performed better than the others when they were planted "away". Thus, we cannot strictly speak about "local adaptation" in this case. This result highlights the importance of considering both criteria in the evaluation of local adaptation.

Population size (Leimu and Fischer 2008) and history (Kawecki and Ebert 2004) are major factors to be considered when looking for local adaptation. Our study population was large, but recent. Indeed, as previously mentioned, the silver fir populations on the three mountains selected in the present study originated from the recolonisation of afforested stands at the end of the nineteenth century. Thus, there may not have been sufficient time for adaptation to fully occur, meaning that differentiation between populations may be due not only to selection but also to a founder effect or to drift. This is actually a common situation among forest trees where populations do not experience stable environments. The absence of local adaptation can also be attributed to the existence of strong inter-annual climate heterogeneity. Indeed, temperatures, precipitation and, consequently, drought stress can vary as much (and sometimes more) between consecutive years at a single site as between stations located 100 m apart in elevation. Finally, the seedlings considered in the present study were only 6 years old, and traits measured at older stages may provide different patterns of variability and adaptation.

According to association studies conducted on adult fir populations from the same mountains (Roschanski et al. 2016), the frequencies of some SNPs may be related to bioclimatic variables, at both the landscape scale (i.e. between sites) and the local scale (i.e. between elevations within sites). Although these findings are based on SNP related to certain candidate genes, they do not provide evidence of the relationship between allele frequencies and adaptive performance, which may explain why we did not detect a generalised pattern of local adaptation. At a larger scale, Brousseau et al. (2016) reported that SNP variability did not provide evidence for differentiation among silver fir populations from high vs low elevations.

5 Conclusion

To our knowledge, the experimental design used in the present study is one of the largest reciprocal transplant experimental approaches ever adopted in plants (at least trees) to analyse the short-distance-scale variability of phenotypic traits and to check for local adaptation. The main identified limit of our experiment is that phenotyping was only realised at the seedling stage. Thus, our results are only one contribution to the full knowledge of fir diversity and adaptation. The seedling stage is however known to be for fir and for most of the tree species, the period of the highest selection pressure and demographic evolution.

The presence of local adaptation at only one low-elevated, dry site, together with the over-performance of the seedlings originating from the sites at the lowest elevations suggests that selection pressures are higher at the dry end of the gradients. On the three studied mountains, severe diebacks of silver fir following the heat wave of 2003 were most frequently observed at low elevations (Cailleret et al. 2014). Seed trees sampled in 2008 at the lowest elevations of each gradient survived these diebacks and may, therefore, have been selected for harsh dry conditions. However, the superior performance of seedlings originating from low elevations remained limited (+ 16% on average for growth and survival performance). Moreover, in the three gradients, inter-family differentiation was higher than inter-population differentiation. Populations showed high phenotypic plasticity and strong genetic effects due to family, which, combined with the absence of local adaptation, provides favourable conditions for coping with ongoing climate change. Fir appears to be an adaptive generalist, and there is no clear benefit to enriching existing mid- or high-elevation populations with genes from low-elevation populations. The main objective will be to maintain high diversity within these populations. At higher elevations, beyond the front edge of the species distribution (i.e. outside the present species range), warming due to climate change will induce favourable conditions for species installation, but fir populations will not be able to fully colonise these environments because their migration rates are lower than the velocity of climate change (Amm et al. 2012). Thus, assisted migration should be recommended only for these places, and preferably using gene pools originating from the lowest and warmest elevations of the gradients that exhibited better performances than other origins. In the present context of global warming and considering (i) the large genetic variability within provenances and (ii) the highest survival rates of the provenances from the lowest elevations, this seed transfer should not generate any risk of frost damages.

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